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Foliar and root applications of the rare sugar tagatose control powdery mildew in soilless grown cucumbers

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ABSTRACT

Powdery mildew is a constant threat to cucumber production in soilless systems requiring the intensive use of chemical fungicides to limit yield losses. However, the toxicity risks for the growers and the food safety issues linked to fungicide residues represent a major concern for crops with a frequent and extended harvest period, like cucumber, and create an urgent need of eco-friendly alternatives. The objective of this study was to assess the effectiveness of foliar and root applications of the rare sugar tagatose, against naturally occurring powdery mildew (*Podosphaera xanthii and Golovinomyces cichoracearum*) on cucumber plants grown in a soilless system under commercial-like greenhouse conditions. Foliar and root applications of tagatose reduced powdery mildew severity and incidence under conditions of low severity in untreated plants, but tagatose and the chemical standard strategy lost their effect in the control of powdery mildew when the disease severity was increasing steadily. The combination of sulphur with foliar applications of tagatose effectively reduced powdery mildew under high severity conditions. Overall, under high disease pressure, foliar and root applications of tagatose were more effective than the standard chemical treatment reducing powdery mildew disease incidence and severity. Foliar application of tagatose directly inhibited *P. xanthii* conidial germination. After root application, tagatose was translocated to the leaves guaranteeing a systemic protection with no accumulation in cucumber fruits. Root applications of tagatose represent a novel strategy for cucumber protection against powdery mildew and reduce fungicide applications.

1. Introduction

In response to the continuous drop in fertile soil and water availability, vegetable production has increased its reliance on soilless agriculture techniques based on hydroponic solution (Sambo et al., 2019). In addition to a more efficient use of water and nutritional resources, hydroponic systems guarantee higher yields, better quality of the products and a continuous production throughout the year (Barbosa et al., 2015; Maucieri et al., 2019). Although root pathogens represent a major threat in hydroponic systems (Lee and Lee, 2015), plants have also to deal with pathogens affecting the aerial part (Elad et al., 1996). Most of the vegetable production under greenhouse conditions suffers from powdery mildew (Elad et al., 1996) that represents one of the most common and severe diseases of cucurbits (Pérez-García et al., 2009). The fungal pathogen *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*, formerly *Sphaerotheca fusca*) is considered the main causal agent of powdery mildew of cucurbits, affecting the aerial part of cucumber and zucchini plants grown under field and greenhouse conditions (Elad et al., 1998; Bettiol et al., 2008). However, powdery mildew can also be caused by *Golovinomyces cichoracearum* var. *cichoracearum (formerly Erysiphe cichoracearum* DC) on cucurbits, as confirmed by the recent taxonomy of Braun and Cook (2012). The presence of the two species can vary depending on the geographic area considered; in France, Netherlands and Great Britain, only mixed infections of *P. xanthii* and *G. cichoracearum* were recorded, while at several locations in Italy, Germany and Slovenia only *P. xanthii* was recorded and there were no locations with an exclusive occurrence of *G. cichoracearum*, but only as a mixed infection with *P. xanthii*. Both powdery mildews were instead

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found in Austria and Switzerland as a unique or mixture species (Křístková et al., 2009). The reason of the different distribution can be attributed to the different climatic conditions (Trecate et al., 2019).

Cucumber (*Cucumis sativus* L.) is one of the most important economic crops belonging to the family of Cucurbitaceae (Weng and Sun, 2011). Powdery mildew infections cause white fungal growth on leaf surfaces, petioles, stems of cucumber plants affecting fruit yield and quality (Elad et al., 1998; Pérez-García et al., 2009). Although the use of resistant cultivars was initially a successful approach, growers had then to rely on traditional fungicides for the control of this fungal pathogen, due to the development of powdery mildew races that overcome plant resistance (Pérez-García et al., 2009). Therefore, application of chemical fungicides is the principal practice to control cucumber powdery mildew, especially under greenhouse conditions (Cerkauskas and Ferguson, 2014). However, the development of fungicide resistance, the toxicity on non-target organisms, the human health risks and environmental issues associated with the use of chemical fungicides have increased the research for eco-friendly alternatives (Pérez-García et al., 2009; Cerkauskas and Ferguson, 2014). Furthermore, cucumber plants grown under greenhouse conditions have a prolonged harvest period (Paradjikovic et al., 2004) and fungicides are repeatedly applied against powdery mildew (Romero et al., 2007) with possible risk of residue accumulation on harvested fruits. The application of eco-friendly products is of particular importance to avoid the accumulation of chemical residues on harvested fruits (Khay et al., 2008).

Rare sugars represent a valuable alternative to chemical fungicides due to their inhibitory properties against phytopathogens (Ohara et al., 2008). Rare sugars are a class of monosaccharides (e.g. sorbose, tagatose, xilulose and xylitol) and their derivatives present in small quantities in the environment (Granström et al., 2004). Although rare sugars are mainly known as low-calorie sweeteners (Levin, 2002; Matsuo et al., 2002), they acquired medical interest as anti-hyperglycaemic (Lu et al., 2008), anticancer (Beerens et al., 2012) and prebiotic molecules (Bertelsen et al., 1999; Vastenavond et al., 2012). Furthermore, the development of novel enzymatic and microbial processes reduced costs of synthesis, extending rare sugar use and study to animal, plant and microbiological systems (Izumori, 2002, 2006). Rare sugars can occur naturally in some higher plants (Fukumoto et al., 2011), such as *Veronica filiformis* (Chari et al., 1981), in crops, such as potato leaves (Weckwerth et al., 2004). Only a limited number of microorganisms is able to produce (e.g. *Exiguobacterium aurantiacum*) (Raichand et al., 2012) or metabolise (e.g. *Bacillus licheniformis* and *Aspergillus niger*) (Hayer et al., 2013; Van der Heiden et al., 2013) rare sugars. Among rare sugars, tagatose is a ketohexose and it is used as a low-calorie sweetener, substitute of sucrose (Kim, 2004). Tagatose was generally recognised as safe by the Food and Drug Administration and it is frequently added to food and beverages as a low-calorie sweetener (Levin, 2002; Kim, 2004). It was reported that tagatose has a prebiotic effect on the human gut (Bertelsen et al., 1999; Vastenavond et al., 2012) and a similar effect was described in grapevine (Perazzolli et al., 2020). The main beneficial effect of tagatose on grapevine was due to the reduction of powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) symptoms (Perazzolli et al., 2020). Tagatose can inhibit the growth of a wide range of phytopathogens (Ohara et al., 2008; Chahed et al., 2020; Mochizuki et al., 2020; Perazzolli et al., 2020). In particular, tagatose can reduce the severity of powdery and downy mildew symptoms on numerous plant hosts (Mochizuki et al., 2020). Thus, tagatose represents a potential alternative to chemical fungicides, with low animal toxicity and low potential risk for the environment (Ohara et al., 2008). Although the mechanism of action was partially investigated in oomycetes (Chahed et al., 2020; Mochizuki et al., 2020; Corneo et al., 2021), the effectiveness of tagatose against cucumber powdery mildew was not characterised in terms of way of application (i.e. foliar and root treatments).

The objectives of this study were to (i) assess, under small-scale greenhouse conditions, the effect of foliar (local) and root (systemic) applications of different dosages of a novel formulation of the rare sugar tagatose on *P. xanthii* germination in soilless grown cucumbers; (ii) determine the most effective dosage, keeping into account beneficial and/or side effects on plant health; (iii) evaluate the effectiveness of the most effective dosage against naturally-occurring powdery mildew on cucumber plants grown in a soilless system under commercial-like greenhouse conditions, and compare it to the standard chemical strategy applied under field conditions in Switzerland.

2. Materials and methods

2.1. Soilless growing system set up

Cucumber plants (cv. Prolog RZ, Rijk Zwaan Italia Srl, Bologna, Italia) were grown in a soilless system under controlled greenhouse conditions at the Fondazione Edmund Mach in San Michele all'Adige (Italy) (dosage trial) and subsequently under commercial-like greenhouse conditions at Cadenazzo (Switzerland) (validation trial). In both dosage and validation trial a substrate composed of perlite (Agrilit 3, 2–5 mm granulometry) and coconut fibres in 1:1 ratio contained in UV-anti ageing coextruded polyethylene grow bags of 20 × 100 cm (grow bag, Agripan C 27 L substrate, Perlite Italiana Srl, Corsico, Italy) was used for plant growth. Grow bags were pre-irrigated up to full capacity with half-strength nutrient Hoagland solution in distilled water (Hoagland and Arnon, 1950) at pH 5.8 ± 0.2 and electrical conductivity (EC) 1.2 ± 0.05 mS/cm. Rockwools of 10 × 10 × 6 cm (Grodan, ROCKWOOL B.V., Roermond, Netherlands) were positioned, equally distanced, on top of each grow bag (four rockwools, for each grow bag in the dosage trial and two in the validation trial).

In the dosage trial eight replicates (plants) were used for each treatment (for treatments see paragraph 2.2.) and distributed over two grow bags positioned on five separate benches, corresponding to five different treatments. Plants were grown under greenhouse conditions at 25 °C with a photoperiod of 14 h light and 75 ± 5% relative humidity (RH). Nutrient solution pH and EC were checked daily using a portable pH/conductivity tester pH/CO1030 (VWR International, Srl, Milano, Italy) and the pH was adjusted using 0.37 M H_2SO_4. Individual rockwools were automatically irrigated with nutrient solution by means of trickle irrigation with eight-fixed irrigations per day. The irrigation was adjusted to maintain a constant daily dripping of 30 ± 5% v/v and after measuring the volume and monitoring pH and EC, the dripping solution was discarded.

In the validation trial (for treatments see paragraph 2.3.), the plant density was 2.5 plant/m^2, the drape system was used as training system and the plants were transplanted in coconut fibre slabs at the four-leaf stage. Heating temperature in the validation trial was set at 21–22 °C and ventilation temperature 0.5 °C above the heating point. The top of the glasshouse was screened during the day in order to prevent high temperature and an excessive transpiration of the plants due to high irradiation. In the validation trial, as a basal protection, all plants were sprayed one week before starting each trial (2015, 2016 and 2017) with the following products: two applications of Armicarb (85% Potassium bicarbonate) at 0.5%, one application of Amistar (22.8% Azoxystrobin) at 0.1%, and one application of Amistar + Thiovit Jet (80% Sulphur) at 0.1%. In the first trial of 2015, no basal protection was supplied and in both trials of 2016, due to the high disease pressure, sprays were alternated with Armicarb in both trials on all plants and Armicarb was applied twice on all plants in the second trial one month after the beginning of the experiment. In the validation trial, the nutrient solution was supplied by means of trickle irrigation with four-fixed irrigations per day and supplementary supplies automatically adjusted depending on the irradiation level. Approximately 25% of the nutrient solution supplied drained off and was reused. The nutrient solution was composed of 15.7 mmol/L NO_3, 0.7 mmol/L P, 10.25 mmol/L K, 0.42 mmol/L Mg, 3.52 mmol/L Ca, 1.5 mmol/L Fe with the addition of a micronutrient standard solution. The pH was regulated between 5.5 and

6.0 and EC was regulated at 1.5 mS/cm at the transplant and between 1.8 and 2.0 mS/cm during the production period.

2.2. Dosage trial

2.2.1. Tagatose treatment

In the dosage trial a formulation of tagatose (F_TAG) (wettable powder containing 80% tagatose w/w (IFP48), provided by Bi-PA nv, Londerzeel, Belgium) was used. Foliar and root applications of F_TAG containing 0.16%, 0.8% or 2.4% tagatose (0.16%, 0.8% and 2.4% F_TAG) and of 0.8% pure tagatose (0.8% TAG) were tested. Eight replicates (plants) were used for each treatment (0.16% F_TAG, 0.8% F_TAG, 2.4% F_TAG, 0.8% TAG and CTRL) and distributed over two grow bags on five separate benches.

2.2.2. Evaluation of the local effect on germination of Podosphaera xanthii conidia

The inoculum was obtained from powdery mildew symptomatic leaves of untreated courgette plants collected in commercial field in Trentino region in 2019. The leaves were kept in a moist chamber at 23 ± 1 °C for 24 h to favour conidia germination and *P. xanthii* identification was carried out by assessing under a light microscope (Eclipse 80i, Nikon, Amsterdam, the Netherlands), the shape (ovoid) of the conidia and the position (lateral) of conidia germination tube (Miazzi et al., 2011). *P. xanthii* was maintained by subsequent inoculations under greenhouse conditions. For inoculum preparation conidia from young leaves carrying fresh sporulation of *P. xanthii* at 14 days post inoculation were brushed gently with a wet paint brush and re-suspended in distilled water (Cappelletti et al., 2017).

To assess the local effect of tagatose on *P. xanthii* conidia germination, fully developed leaves were collected from untreated cucumber plants part of the dosage trial (32 and 59 days after sowing). Leaves were surface sterilised by incubation in 0.5% hypochlorite for 3 min, and rinsed three times in sterile water for 5 min under orbital shaking at 60 rpm. Leaf discs (19 mm diameter) were cut out and placed (adaxial surface uppermost) on wet sterilised filter paper (three foils) in Petri dishes (Cappelletti et al., 2017), and then homogenously sprayed with tagatose (0.16% F_TAG, 0.8% F_TAG, 2.4% F_TAG, 0.8% TAG and CTRL) using a 30 mL plastic hand sprayer. Treated leaf discs were let dry under a chemical hood for 20 min and sprayed with a suspension of *P. xanthii* conidia (1×10^5 conidia/mL) in distilled water using a 30 mL plastic hand sprayer. Control leaf discs (CTRL) were sprayed with distilled water. Inoculated leaf discs were incubated for 48 h at 23 ± 1 °C with a RH of 99% and 16 h photoperiod to promote conidial germination (Cappelletti et al., 2017). Conidia were removed from the leaf disc using a piece of transparent adhesive tape and stained with a drop of Cotton Blue staining solution (Peries, 1962). The percentage of germinated conidia was assessed by counting under a light microscope (Eclipse 80i, Nikon). Three replicates of three discs were assessed for each treatment and the experiment was carried out twice. The germination of 50 randomly selected conidia was assessed for each disc. Conidia were scored as germinated when their germ tube length was greater than their lateral radius (Pertot et al., 2007).

2.2.3. Evaluation of the systemic effect on germination of Podosphaera xanthii conidia and side effect on plant growth

To assess the systemic effect of the root application of tagatose on *P. xanthii* conidia germination, starting from the 7th true leaf stage, tagatose was applied once a week for four consecutive weeks. Tagatose was dissolved in fresh nutrient solution and added to the substrate (43 mL/plant, corresponding to the volume of individual irrigations). Control plants were treated with the same volume of nutrient solution. Before each application, the irrigation was interrupted overnight for 12 h to maximise tagatose absorption (Jermini et al., 2019).

The number of total leaves was assessed one-day after receiving two and four root applications in order to monitor plant growth as well as plant stress in response to tagatose application.

One-day after receiving two and four root applications, half apical leaf of three replicates (plants) per treatment (0.16% F_TAG, 0.8% F_TAG, 0.8% TAG and CTRL) was collected. Leaves were surface sterilised and leaf discs were inoculated with *P. xanthii* conidia (1×10^5 conidia/mL) as described above, in order to assess the conidial germination. Three replicates of three discs were assessed for each treatment and the germination of 50 randomly selected conidia was assessed for each disc.

2.2.4. Evaluation of the systemic effect of tagatose on chlorophyll, flavonol and anthocyanin content

Chlorophyll, flavonol and anthocyanin content was assessed with a Dualex Scientific optical leaf clip sensor (Force-A, Orsay, France) (Cerovic et al., 2012). Measurements were carried out on 16 basal and 24 apical leaves distributed over eight plants one-day after receiving two root applications and on eight basal and 12 apical leaves distributed over four plants per treatment (0.16% F_TAG, 0.8% F_TAG, 0.8% TAG and CTRL) one-day after receiving four root applications. Chlorophyll, flavonol and anthocyanin content of individual leaves represented the average of three randomly distributed measurements on the adaxial side of the leaf avoiding main veins (Agati et al., 2016).

2.2.5. Tagatose quantification in plant tissues

Leaf samples were collected one-day after receiving two and four root applications. Three replicates (plants) were sampled per treatment (0.16% F_TAG, 0.8% F_TAG, 0.8% TAG and CTRL). Half of a basal and half of an apical leaf were collected from each plant and immediately frozen in liquid nitrogen for subsequent quantification of tagatose content. Cucumber fruits were collected at plant maturity, blend (using a conventional kitchen blender) and frozen at −20 °C. Three replicates (plants) were sampled per treatment (0.16% F_TAG, 0.8% F_TAG, 0.8% TAG and CTRL). The tagatose content was assessed by ion chromatography as previously described (Cataldi et al., 2000) and it was expressed as quantity of tagatose per unit of fresh tissue weight g/kg, using a calibration curve of 98.5% pure tagatose (Sigma-Aldrich, St. Louis, MO, USA) dissolved in ultrapure water within a range between 0.2 and 25 mg/L. Briefly "samples were diluted 50 fold in ultrapure water, filtered through a 0.45 μm PTFE membrane (Sartorius, Goettingen, Germany) and analysed with an ionic chromatograph ICS 5000 (Dionex-Thermo Scientific, Waltham, MA, USA), equipped with an autosampler, a quaternary gradient pump, a column oven and a pulsed amperometric detector with a gold working electrode and a palladium counter electrode. The separation was obtained by injecting 5 μl of diluted sample onto a CarboPac PA200 3 × 250 mm analytical column (Dionex-Thermo Scientific, Waltham, MA, USA), preceded by a CarboPac PA200 3 × 50 mm guard column (Dionex-Thermo Scientific), with a KOH gradient (from 1 to 100 mM) at 0.4 mL/min flow rate" (Perazzolli et al., 2020).

2.3. Validation trial

2.3.1. Tagatose treatment, chemical treatment and control

The most effective dosage of F_TAG (0.8% F_TAG) in both foliar and root application of the dosage trial was tested in the validation trial. Two independent trials (spring and autumn trials) for three consecutive years (2015, 2016 and 2017) were carried out. For each experiment, a randomized complete block design with three replicates of eight plants in 2015 and five replicates of eight plants in 2016–2017 per each treatment was used. In 2015 the plot area for each treatment was 50 m^2. Each plot was divided in three subplots with eight plants/subplot, placed in the central row of the plot, on which the disease assessment was carried out. In 2016 and 2017 plots had an area of 86 m^2 and each plot was divided in five subplots of eight plants on which the disease assessment was carried out.

The effect of 0.8% F_TAG in the reduction of powdery mildew was compared to that of untreated control plants (CTRL) and standard

strategies for powdery mildew control (chemical treatment; Table 1), complying with the chemical standard strategy used in commercial cucumber production under field conditions in Switzerland. Details of the volume of mixture applied for each product (L/ha) are reported in Table 1.

In the spring trial of 2015 three applications of Amistar (22.8% Azoxystrobin) at 0.1%, followed by three applications of Stroby (50% Kresoxim-Methyl) at 0.02% and Forum (13.9% Dimethomorph) at 0.1% were applied (Table 1). In the autumn trial of 2015 the chemical treatment consisted of one application of Amistar at 0.1%, followed by one application of Verita (66.7% Fosetyl Aluminium + 4.4% Fenamidone) at 2.5 kg/ha and Thiovit Jet (80% Sulphur) at 0.1%.

In the spring trial of 2016 the chemical treatment consisted of two applications of Amistar at 0.1%, followed by two applications of Verita at 2.5 kg/ha and Thiovit Jet at 0.1%, two applications of Stroby at 0.02% and Forum at 0.1% and a final application of Verita at 2.5 kg/ha and Thiovit Jet at 0.1%. In the autumn trial of 2016 the chemical treatment consisted of three consecutive applications of Amistar at 0.1%, followed by two applications of Armicarb (85% Potassium bicarbonate) at 0.5% and two applications of Verita at 2.5 kg/ha and Thiovit Jet at 0.1%.

In the spring trial of 2017 the chemical treatment consisted of two applications of Amistar at 0.1% and Thiovit Jet at 0.1%, followed by two applications of Armicarb at 0.5% and three applications of Verita 2.5 kg/ha and Thiovit Jet at 0.1%. Finally, in the autumn trial of 2017 the chemical treatment consisted of two applications of Amistar at 0.1% and Thiovit Jet at 0.1%, followed by four applications of Verita at 2.5 kg/ha and Thiovit Jet at 0.1% (Table 1).

Chemical treatment and F_TAG treatments were applied on plants with the same timing. In 2015 and 2016 F_TAG was applied as foliar spray, while in 2017 F_TAG was applied in nutrient solution of the fertigation system. Due to the high disease pressure in 2016, 0.1% Thiovit Jet (80% Sulphur) was used in tank mixture with F_TAG as applied to CTRL plants as control. The fungicides (chemicals and F_TAG) were applied using a motorized knapsack sprayer Birchmeier M 225-20 equipped with two mist nozzles 1.0 mm and at a pressure of 13 bar when plants were shorter than 50–60 cm, and after with a vertical boom sprayer with air assistance (Turbo M.A.B.) equipped with eight nozzles DTK 120-2 yellow Lechler and at a pressure of 2.5 bar. In 2017 F_TAG was applied via drip irrigation system (Jermini et al., 2019) by isolating the treated plants to avoid the transfer of the fungicide via drip irrigation in the other treatments.

The chemigation-system consisted of a volumetric pump that guaranteed a constant pressure during the injection and a low pressure flow rate. The system was calibrated before the experiment; the optimal flow rate and the amount of water required were pre-determined and kept constant at each application to guarantee a uniform distribution of the product. F_TAG injections were carried out as close as possible to the crop. Before each F_TAG application, the last fixed irrigation in the evening was suspended so as to have a period of at least 12 h without water supply. This procedure induces a low water stress in the plants allowing to maximise tagatose absorption (Jermini et al., 2019), which was injected during the first irrigation in the morning of the day after. The product was added using tap water without fertilizers to avoid interactions between the product and the nutrient solution.

2.3.2. Evaluation of the effect of tagatose against cucumber powdery mildew in the validation trial

Symptoms of naturally occurring powdery mildew (*P. xanthii and G. cichoracearum*) were assessed visually each week by randomly checking four leaves per plant (one basal, two central and one apical), respectively. Disease severity was assessed as a percentage of infected leaf area covered by white powdery mildew conidia and mycelia, and the disease incidence was calculated as percentage of infected leaves showing white powdery mildew sporulation, according to the guidelines of the European and Mediterranean Plant Protection Organization (EPPO, 2004b). To evaluate the cumulative impact of powdery mildew throughout the entire season, "the development of the disease in terms of severity and incidence was assessed as the area under the disease progress curve (AUDPC) using the following formula:

$$\mathrm{AUDPC} = \sum \frac{(X_i + X_{i+1})}{2} + (t_{i+1} - t_i)$$

where X_i corresponds to either disease severity or incidence (%) at assessment i, X_{i+1} corresponds to either the severity or incidence (%) at subsequent assessment $i + 1$, and $(t_{i+1} - t_i)$ corresponds to the number of days between the two consecutive assessments" (Nesler et al., 2015). In addition, downy mildew (*Pseudoperonospora cubensis*) severity and incidence were assessed in the autumn trial of each year according to the guidelines of (EPPO, 2004a) due to the control spectrum of tagatose on this pathogen (Mochizuki et al., 2020).

2.4. Statistical analyses

Data were analysed with PAST 4.03 (Hammer et al., 2001) and normal distribution (Shapiro-Wilk test, $P > 0.05$) and variance homogeneity (Levene's test, $P > 0.05$) were validated. When both assumptions were satisfied, analysis of variance (ANOVA) with the Tukey post-hoc test ($P \leq 0.05$) was carried out to detect significant differences among treatments (conidial germination after foliar application in the dosage trial). When data were not normally distributed, they were Log_{10} transformed (conidial germination after root application in the dosage trial and powdery mildew severity and incidence assessed in 2015 validation trial) or square-root transformed (tagatose content after root application in the dosage trial). When parametric assumptions were not satisfied, the Kruskal-Wallis test was used to detect significant differences among treatments ($P \leq 0.05$) (total number of leaves, chlorophyll, flavonol and anthocyanin content after root application in the dosage trial, powdery mildew severity and incidence in 2016 and 2017 trials, AUDPC values and downy mildew severity and incidence) with the Bonferroni correction (Zar, 1996). Zero values were replaced with a random value between zero and the detection limit for the tagatose content analysis (Chitarrini et al., 2017).

Correlations between chlorophyll and anthocyanin content were tested using the ordinary least square linear regression analysis ($P \leq 0.05$).

3. Results

3.1. Tagatose applications reduce Podosphaera xanthii symptoms and increase chlorophyll content of cucumber leaves in the dosage trial

Conidial germination was lower in 0.8% F_TAG-, 2.4% F_TAG- and 0.8% TAG-treated leaf discs compared to CTRL leaf discs after foliar application, while it was comparable in CTRL and 0.16% F_TAG-treated leaf discs (Fig. 1). In particular, conidial germination of 0.8% TAG-treated leaf discs was comparable to that of 0.16% F_TAG- and 0.8% F_TAG-treated leaf discs and higher than that of 2.4% F_TAG-treated leaf discs.

Conidial germination was lower in 0.16% F_TAG-, 0.8% F_TAG- and 0.8% TAG-treated plants compared to CTRL plants after two and four root applications (Fig. 2a) and it was comparable among the different tagatose treatments. The total number of leaves was lower in 2.4% F_TAG-treated plants compared to the other treatments (CTRL, 0.16% F_TAG, 0.8% F_TAG and 0.8% TAG; Fig. 2b) and this dosage was therefore discarded due to its negative effects on plant growth. Tagatose content in basal and apical leaves was higher in 0.8% F_TAG-treated plants compared to CTRL, 0.16% F_TAG- and 0.8% TAG-treated plants after two and four root applications, with the exception of the apical leaves after two applications (Fig. 3). Tagatose content was higher in basal leaves of 0.8% TAG-treated plants compared to CTRL plants at both time points (Fig. 3). However, tagatose content in the fruits was

Table 1
Application date and fungicide spray interval, expressed as days after previous application, commercial name, concentration of active ingredients, dose rate used and volume applied in 2015, 2016 and 2017 in the chemical treatments used in the validation trial [spring (1) and autumn (2) trials].

Trial/ year	Application date	Days after previous applications	Fungicide			
			Commercial name	Concentration of active ingredient	Dose rate	Volume mixture applied (L/ ha)
1/2015	2.04	0	Amistar	22.8% Azoxystrobin	0.1%	306
	13.04	11	Amistar	22.8% Azoxystrobin	0.1%	383
	30.04	17	Amistar	22.8% Azoxystrobin	0.1%	689
	14.05	14	Stroby	50% Kresoxim-Methyl	0.02%	766
			Forum	13.9% Dimethomorph	0.1%	
	29.05	15	Stroby	50% Kresoxim-Methyl	0.02%	766
			Forum	13.9% Dimethomorph	0.1%	
	12.06	14	Stroby	50% Kresoxim-Methyl	0.02%	766
			Forum	13.9% Dimethomorph	0.1%	
2/2015	13.08	3	Amistar	22.8% Azoxystrobin	0.1%	613
	20.08	7	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	689
			Thiovit Jet	80% Sulphur	0.1%	
1/2016	26.04	19	Amistar	22.8% Azoxystrobin	0.1%	666
	6.05	10	Amistar	22.8% Azoxystrobin	0.1%	914
	25.05	13	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1110
			Thiovit Jet	80% Sulphur	0.1%	
	2.06	13	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1070
			Thiovit Jet	80% Sulphur	0.1%	
	16.06	13	Stroby	50% Kresoxim-Methyl	0.02%	979
			Forum	13.9% Dimethomorph	0.1%	
	24.06	13	Stroby	50% Kresoxim-Methyl	0.02%	1044
			Forum	13.9% Dimethomorph	0.1%	
	7.07	13	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1070
			Thiovit Jet	80% Sulphur	0.1%	
2/2016	5.08	7	Amistar	22.8% Azoxystrobin	0.1%	548
	15.08	10	Amistar	22.8% Azoxystrobin	0.1%	548
	23.08	8	Amistar	22.8% Azoxystrobin	0.1%	548
	30.08	7	Armicarb	85% Potassium bicarbonate	0.5%	1027
	3.09	4	Armicarb	85% Potassium bicarbonate	0.5%	1118
	9.09	6	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1110
			Thiovit Jet	80% Sulphur	0.1%	
	23.09	14	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	940
			Thiovit Jet	80% Sulphur	0.1%	
1/2017	14.04		Amistar	22.8% Azoxystrobin	0.1%	274
			Thiovit Jet	80% Sulphur	0.1%	
	25.04	11	Amistar	22.8% Azoxystrobin	0.1%	339
			Thiovit Jet	80% Sulphur	0.1%	
	28.04	3	Armicarb	85% Potassium bicarbonate	0.5%	979
	1.05	3	Armicarb	85% Potassium bicarbonate	0.5%	979
	12.05	11	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1201
			Thiovit Jet	80% Sulphur	0.1%	
	25.05	13	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1253
			Thiovit Jet	80% Sulphur	0.1%	
	12.06	18	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1175
			Thiovit Jet	80% Sulphur	0.1%	
2/2017	9.08		Amistar	22.8% Azoxystrobin	0.1%	457
			Thiovit Jet	80% Sulphur	0.1%	
	16.08	7	Amistar	22.8% Azoxystrobin	0.1%	744
			Thiovit Jet	80% Sulphur	0.1%	
	21.08	5	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	809
			Thiovit Jet	80% Sulphur	0.1%	
	29.08	8	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1070
			Thiovit Jet	80% Sulphur	0.1%	
	8.09	10	Verita	66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	1214
			Thiovit Jet	80% Sulphur	0.1%	
	22.09	14	Verita			1175

(*continued on next page*)

Table 1 (*continued*)

Trial/ year	Application date	Days after previous applications	Fungicide			
			Commercial name	Concentration of active ingredient	Dose rate	Volume mixture applied (L/ ha)
				66.7% Fosetyl Aluminium + 4.4% Fenamidone	2.5 kg/ ha	
			Thiovit Jet	80% Sulphur	0.1%	

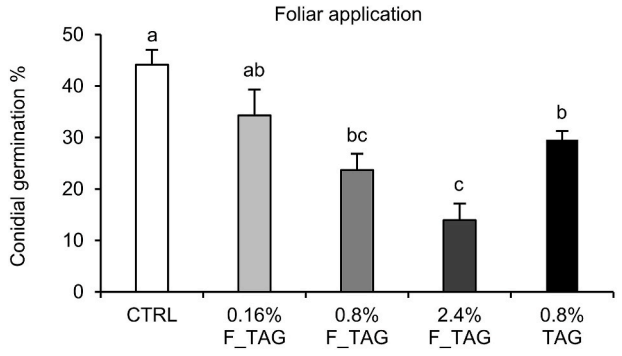

Fig. 1. Effect of tagatose foliar application on the germination of *Podosphaera xanthii* conidia. The percentage of germinated *Podosphaera xanthii* conidia was assessed on surface-sterilised leaf discs sprayed with 0.16% (0.16% F_TAG), 0.8% (0.8% F_TAG) or 2.4% (2.4% F_TAG) tagatose as formulated product or 0.8% pure tagatose (0.8% TAG). Control discs (CTRL) were sprayed with distilled water. Bars represent mean conidial germination percentage and standard error values of six replicates (dishes with three leaf discs each). Different letters indicate significant differences among treatments, according to the Tukey test ($P \leq 0.05$).

below the detection limit (<0.1 g/kg) in CTRL, 0.16% F_TAG-, 0.8% F_TAG- and 0.8% TAG-treated plants.

Chlorophyll content was higher in basal leaves of 0.8% F_TAG-treated plants compared to CTRL and 0.16% F_TAG-treated plants after two root applications and it was higher in apical leaves of 0.8% F_TAG-treated plants compared to the other treatments (CTRL, 0.16% F_TAG and 0.8% TAG) after four root applications (Fig. A1a). Flavonol content was higher in the basal leaves of 0.8% F_TAG-treated plants compared to 0.16% F_TAG-treated plants after two root applications (Fig. A1b). Anthocyanin content was lower in the basal leaves of 0.8% F_TAG-treated plants compared to the other treatments (CTRL, 0.16% F_TAG and 0.8% TAG) after two root applications (Fig. A1c). Moreover, a negative correlation was found between chlorophyll and anthocyanin content in the basal ($P < 0.0001$, $r^2 = 0.61$) and apical ($P < 0.0001$, $r^2 = 0.66$) leaves after two root applications (Fig. A2a-b) and in the basal ($P < 0.0001$, $r^2 = 0.71$) and apical ($P < 0.0001$, $r^2 = 0.74$) leaves after four root applications (Fig. A2c-d).

3.2. Foliar and root applications of tagatose reduce powdery mildew in the validation trial

The three seasons were characterised by different levels of disease pressure, as indicated by different severity levels, and this allowed a comparison of efficacy of tagatose under a wide range of conditions (Fig. 4). While in 2015 powdery mildew severity in the CTRL plants was below 2 and 6%, in the spring and autumn trial respectively, in 2016 powdery mildew severity reached levels above 80% and in 2017 it reached a maximum of about 40 and 20% respectively in the spring and autumn trial. In the trials, the chemical standard strategies were able to control the disease, although with some difficulties under high disease pressure (high severity), caused by the increase of inoculum due to the presence of the untreated control within the same greenhouse. Although powdery mildew disease pressure was low in 2015, as indicated by low disease severity of CTRL plants (Fig. 4a–b), foliar applications of 0.8% F_TAG reduced powdery mildew severity and incidence in the spring trial and the incidence in the autumn trial compared to CTRL plants (Fig. 4a–b). The reduction of powdery mildew incidence in 0.8% F_TAG-treated plants was comparable to that in the chemical treatment and the effect of 0.8% F_TAG lasted throughout the spring trial (Fig. 4a). Foliar applications of 0.8% F_TAG or chemical treatment reduced powdery mildew incidence in the initial phase of the autumn trial, but when the disease severity increased steadily in the second phase of the trial (18 days after first survey, DAS), neither 0.8% F_TAG nor the chemical treatment decreased powdery mildew incidence or severity (Fig. 4b).

In 2016, disease pressure was high (high severity) and powdery mildew severity increased rapidly through the season (Fig. 4c–d). Foliar applications of 0.8% F_TAG reduced powdery mildew severity and incidence compared to CTRL plants in the spring trial, while the chemical treatment reduced the severity, but not the incidence (Fig. 4c). In the autumn trial, 0.8% F_TAG and the chemical treatment did not reduce powdery mildew incidence compared to CTRL plants, due to a

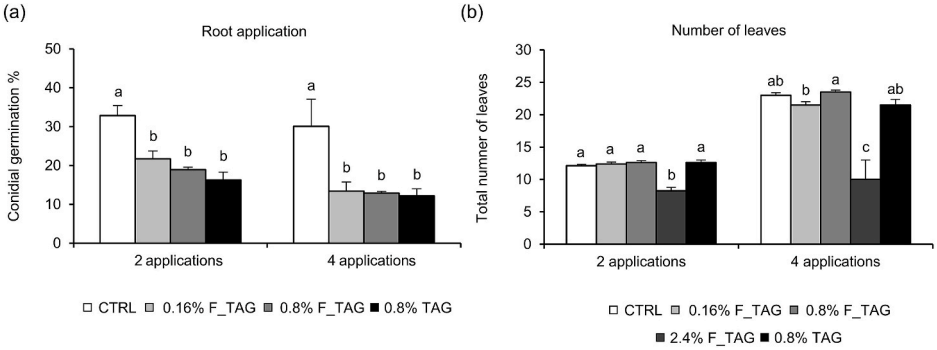

Fig. 2. Effect of tagatose root application on the germination of *Podosphaera xanthii* conidia and on total number of leaves. The percentage of germinated *Podosphaera xanthii* conidia (a) was assessed on surface-sterilised leaf discs obtained from cucumber plants collected one-day after receiving two (2 applications) and four (4 applications) root applications of 0.16% (0.16% F_TAG) and 0.8% (0.8% F_TAG) tagatose as formulated product or 0.8% pure tagatose (0.8% TAG). Control plants (CTRL) were treated with Hoagland solution. Bars represent mean conidial germination percentage and standard error values of three replicates (dishes with three leaf discs each). For each time point, different letters indicate significant differences among treatments, according to the Tukey test ($P \leq 0.05$). The total number of leaves (b) was assessed one-day after receiving 2 applications and 4 applications of 0.16% (0.16% F_TAG), 0.8% (0.8% F_TAG) or 2.4% (2.4% F_TAG) tagatose as formulated product or 0.8% pure tagatose (0.8% TAG). Bars represent mean and standard error values of eight (after 2 applications) and four (after 4 applications) replicates (plants). For each time point, different letters indicate significant differences among treatments, according to the Kruskal-Wallis test ($P \leq 0.05$).

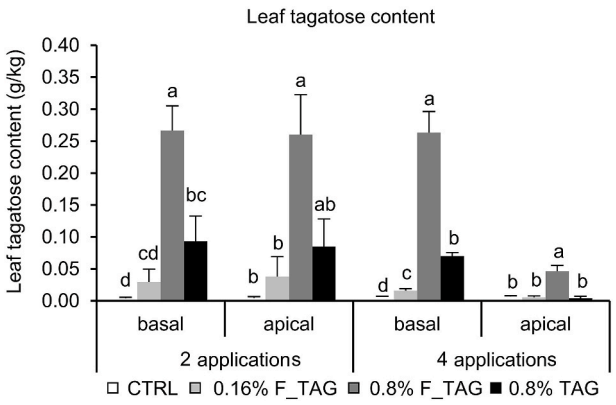

Fig. 3. Tagatose content in cucumber leaves after root application. Tagatose content was measured in the basal and apical leaves of cucumber plants one-day after receiving two (2 applications) and four (4 applications) root applications of 0.16% (0.16% F_TAG) and 0.8% (0.8% F_TAG) tagatose as formulated product or 0.8% pure tagatose (0.8% TAG). Control plants (CTRL) were treated with Hoagland solution. Bars represent mean and standard error values of tagatose content of three replicates (plants). For each time point, different letters indicate significant differences among treatments, according to the Tukey test ($P \leq 0.05$). The statistical analyses were performed separately for basal and apical leaves at each time point.

fast increase of powdery mildew severity (Fig. 4d). However, 0.8% F_TAG decreased powdery mildew severity compared to CTRL plants, even when the disease severity started steadily increasing in the CTRL plants (12 DAS), while the chemical treatment lost its protective effect more rapidly. Foliar applications of 0.8% F_TAG reduced AUDPC of disease severity compared to CTRL plants in the spring and autumn trial of 2016 and AUDPC calculated for the disease incidence in the spring trial of 2016 and in the autumn trial of 2015 (Table 2). Foliar applications of 0.8% F_TAG decreased downy mildew severity and incidence in the autumn trial of 2015 even under high level of disease incidence (Table 3). In 2016 foliar applications of 0.8% F_TAG reduced downy mildew severity, but not incidence throughout the autumn trial with a stronger effect than the chemical treatment (Table 3).

In 2017 root applications of 0.8% F_TAG decreased powdery mildew incidence compared to CTRL plants in the initial phase of the spring trial (up to 21 DAS), with a stronger effect than the chemical treatment (Fig. 4e). Root applications of 0.8% F_TAG decreased powdery mildew severity throughout the spring trial even under high disease severity conditions, while the chemical treatment decreased the disease incidence only until the third survey (14 DAS) and to a lesser extent than 0.8% F_TAG. In the autumn trial, root applications of 0.8% F_TAG decreased powdery mildew severity compared to CTRL plants at some time points, while the chemical treatment was ineffective (Fig. 4f). Root applications of 0.8% F_TAG reduced AUDPC calculated for the disease severity and incidence only in the spring trial (Table 2). In 2017 root application of 0.8% F_TAG reduced downy mildew severity compared to CTRL plants throughout the autumn trial and 0.8% F_TAG was generally more effective than the chemical treatment (Table 3), while it was

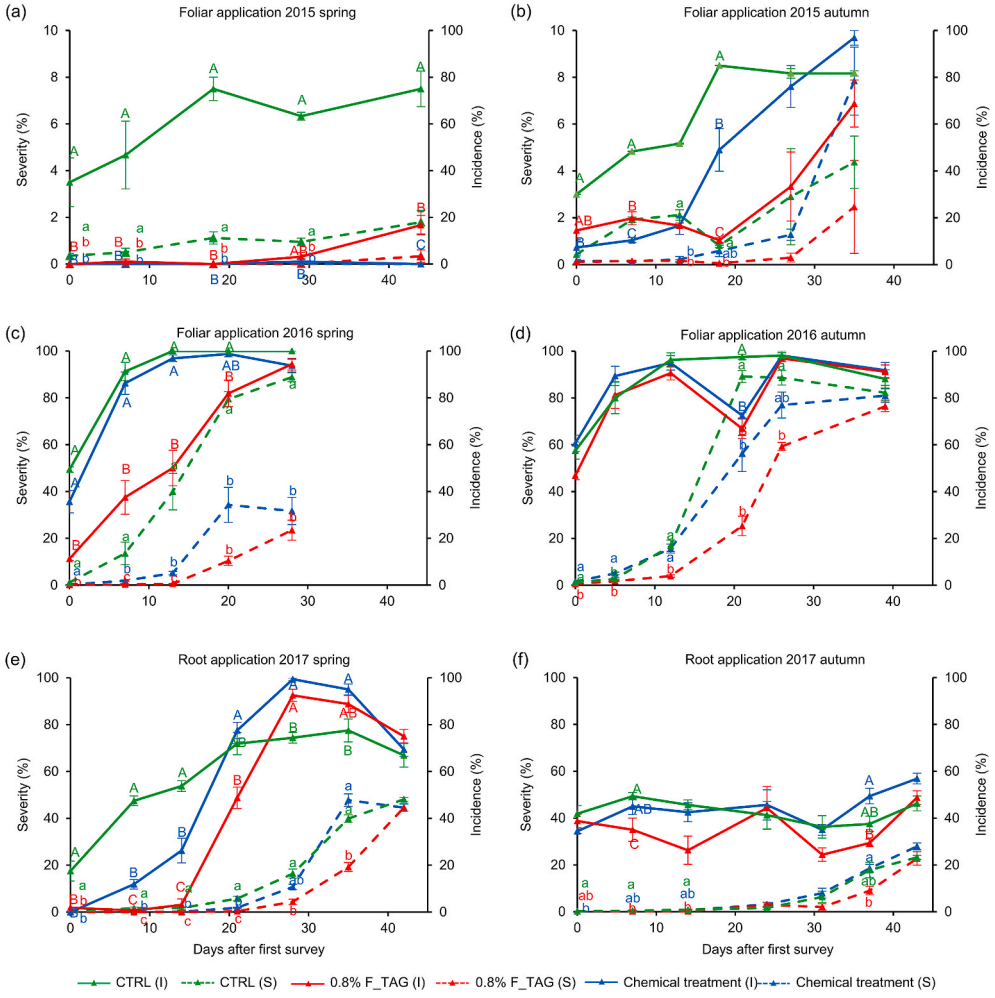

Fig. 4. Effect of tagatose foliar and root application against powdery mildew on cucumber plants. Powdery mildew severity (S, left Y-axis) (dashed line) and incidence (I, right Y-axis) (solid line) were evaluated on cucumber plants after application of 0.8% tagatose as formulated product (0.8% F_TAG, red). Two independent trials were carried out each year (spring trial and autumn trial) and tagatose was applied as foliar application (in 2015, a-b), foliar application in combination with sulphur (in 2016, c-d) and root application (in 2017, e-f). As control, plants were left untreated (CTRL) or treated with chemical products commonly used for powdery mildew control (chemical treatment, blue) (refer to Table 1 for the list of treatments). The mean severity and incidence and standard error of three (2015) and five (2016 and 2017) replicates (each replicate composed by eight plants) are presented for each treatment. For each time point, different uppercase and lowercase letters indicate significant differences of disease incidence and severity respectively, according to the Tukey test ($P \leq 0.05$, 2015) or to the Kruskal-Wallis test ($P \leq 0.05$, 2016–2017). The statistical analyses were performed separately at each time point. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Area under the disease progress curve (AUDPC) of cucumber powdery mildew.

Year	Treatment	AUDPC on severity		AUDPC on incidence	
		Spring	Autumn	Spring	Autumn
2015	CTRL	43.8 ± 1.8a	73.4 ± 21.2	2753.3 ± 73.9a	2319.2 ± 243.9a
	0.8% F_TAG	2.8 ± 2.1ab	15.1 ± 9.7	175.0 ± 46.9ab	902.6 ± 160.8b
	Chemical treatment	0.1 ± 0.1b	49.0 ± 6.9	13.5 ± 13.5b	1561.5 ± 161.8ab
2016	CTRL	1302.9 ± 107.0a	2110.6 ± 58.5a	2565.9 ± 28.5a	3532.2 ± 70.9
	0.8% F_TAG	177.7 ± 32.2b	1250.6 ± 59.6b	1599.7 ± 137.8b	3262.8 ± 97.4
	Chemical treatment	430.3 ± 77.0b	1772.8 ± 110.8ab	2430.6 ± 59.4a	3435.6 ± 74.0
2017	CTRL	626.5 ± 34.6a	242.3 ± 40.4	2552.2 ± 69.5a	1830.0 ± 103.6
	0.8% F_TAG	324.3 ± 22.6b	163.7 ± 29.5	1904.7 ± 67.7b	1461.9 ± 121.0
	Chemical treatment	580.3 ± 30.8a	282.4 ± 24.7	2399.7 ± 49.9a	1878.8 ± 119.7

AUDPC was calculated for powdery mildew severity and incidence on leaves of cucumber plants after foliar (2015 and 2016) or root (2017) application of 0.8% tagatose as formulated product (0.8% F_TAG). Two independent trials were carried out each year (spring and autumn) and F_TAG was combined with 0.1% Thiovit Jet (80% Sulphur) in the 2016 trial. As control, plants were left untreated (CTRL) or treated with chemical products commonly used for powdery mildew control (chemical treatment). The chemical treatment (Table 1) consisted of: three treatments of Amistar at 0.1% (AM) and three applications of Stroby at 0.02% (ST) and Forum at 0.1% (FO) in the spring trial of 2015; one application of AM, one application of Verita at 2.5 kg/ha (VE) and Thiovit Jet at 0.1% (TH) in the autumn trial of 2015; two applications of AM, followed by two applications of VE and TH, two applications of ST and FO and a final application of VE and TH in the spring trial of 2016; three applications of AM, followed by two applications of Armicarb at 0.5% (AR) and two applications of VE and TH in the autumn trial of 2016; two applications of AM and TH, followed by two applications of AR and three applications of VE and TH in the spring trial of 2017; two applications of AM and TH, followed by four applications of VE and TH in the autumn trial of 2017. Mean and standard error values of three (2015) and five (2016 and 2017) replicates (eight plants each) are reported for each treatment. For each trial, different letters indicate significant differences among treatments, according to the Kruskal-Wallis ($P \leq 0.05$). Letters were omitted for time points with no significant differences among treatments.

ineffective and did not reduce the incidence of the disease, that at some time points was higher than in the CTRL plants.

4. Discussion and conclusions

The use of tagatose for the control of powdery mildew represents the opportunity of producing cucumbers reducing the use chemical fungicide residues (Mochizuki et al., 2020), relying on the protection of a safe substance. Under conditions of low severity (2015), foliar applications of 0.8% F_TAG reduced powdery mildew incidence to the same, or higher, extent as the chemical treatment, however when the infection level increased rapidly (e.g. autumn trial 2015), both 0.8% F_TAG and the chemical treatment lost their effect. When powdery mildew severity was high in the CTRL plants, as observed in the trial of 2016, good efficacy can be reached combining 0.8% F_TAG with foliar applications of sulphur. At more advanced plant growth stages, disease pressure tends to increase and frequent fungicide applications are often required to effectively control powdery mildew when reduced risk fungicides (e.g. potassium bicarbonate) and chemical fungicide strategies are used (Cerkauskas and Ferguson, 2014). Foliar applications of tagatose and of the formulated product (0.8% and 2.4% F_TAG) decreased the germination of *P. xanthii* conidia on leaf discs, demonstrating a local effect against powdery mildew. Tagatose can inhibit the growth of numerous phytopathogens (Ohara et al., 2008; Chahed et al., 2020; Mochizuki et al., 2020; Perazzolli et al., 2020) and can be metabolised only by a very limited number of microorganisms (Van der Heiden et al., 2013). Furthermore, tagatose did not modulate the expression pattern of defence-related genes, neither in cucumber plants in the absence of a pathogen inoculation nor in *Arabidopsis thaliana* plants with or without downy mildew inoculum (Mochizuki et al., 2020). Although further biochemical and enzymatic validations are required, tagatose could directly inhibit powdery mildew enzymes involved in sugar metabolism, as described in the case of *Hyaloperonospora arabidopsidis* fructokinase and phosphomannose isomerase (Mochizuki et al., 2020).

Tagatose also demonstrated a systemic protection in the control of powdery mildew. Root application of 0.8% F_TAG reduced powdery mildew as observed for the foliar application. The root application reduced powdery mildew when severity was low in the CTRL plants, but it was ineffective in the more advanced stages of plant growth when disease severity increased rapidly. Overall, the root application of 0.8% F_TAG controlled the disease to a higher extent than the chemical treatment and even in the autumn trial of 2017 it decreased powdery mildew severity at some time points, while the chemical treatment was ineffective. Systemic fungicides represent a key strategy to effectively control powdery mildew (Erwin, 1973; McGrath, 2015) and root application can be more effective in the control of cucurbitaceae powdery mildew than foliar application, as previously observed in the case of silicon (Liang et al., 2005; Dallagnol et al., 2015). The systemic effect of tagatose was possibly due to a translocation of tagatose from the roots to the leaves, since tagatose was found in both apical and basal leaves of cucumber plants after root application. The mobility of systemic fungicides can occur either via the xylem and cell walls (apoplastic mobility) or via the plasmodesmata from cell to cell (symplastic mobility) that involves uptake and distribution through the phloem (Oliver and Hewitt, 2014). Furthermore, root application of 0.8% F_TAG led to an increase in the leaf chlorophyll content, which can be regarded as a beneficial effect of tagatose on plant health (Macías-Rodríguez et al., 2018) and by a decrease in the leaf anthocyanin content indicating the absence of plant nitrogen deficiency (Taiz and Zeiger, 2002). Tagatose exhibited also local and systemic effect against cucumber downy mildew and it reduced downy mildew severity in the different trials, confirming the local effect previously described (Mochizuki et al., 2020) and demonstrating the systemic effectiveness upon root application.

While the effect of foliar applications of tagatose in the control of phytopathogens was previously described (Mochizuki et al., 2020), in this study we reported the systemic effect of tagatose in the control of powdery and downy mildew of cucumber by root application. Thus, the root application of tagatose for plant protection could represent an advantage in terms of reduction of the working hours and reduction of the health risks associated with the exposure to fungicide due to inhaling of dust particles after spraying (Amoatey et al., 2020). Tagatose content in the apical leaves was possibly diluted during plant growth and no tagatose residues were found in cucumber fruits making them safe for human consumption. Moreover, considering that tagatose is registered as a low-calorie sweetener (Bertelsen et al., 1999), the presence of negligible quantity of tagatose in cucumber fruits would not represent any risk for human consumption.

In conclusion, tagatose controlled cucumber powdery and downy mildew and reduced the diseases to a higher extent than the chemical treatment under high disease pressure, especially when combined with sulphur. Tagatose acts as a systemic fungicide and is a promising product for cucumber protection in soilless systems with low dosages of chemical fungicides and with reduced risks for growers under commercial-like greenhouse conditions.

Author contributions

PEC: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Project Administration. MJ: Conceptualization, Methodology,

Table 3
Effect of tagatose foliar and root application against downy mildew on cucumber plants.

Year	Days after first downy mildew survey	Days after first powdery mildew survey	Severity%			Incidence%		
			CTRL	0.8% F_TAG	Chemical treatment	CTRL	0.8% F_TAG	Chemical treatment
2015	0	27	62.1 ± 6.9a	2.4 ± 1.5b	14.8 ± 9.1ab	100.0 ± 0.0	75.0 ± 15.7	96.9 ± 3.1
	8	35	65.8 ± 3.4a	3.3 ± 2.7b	24.8 ± 9.2ab	100.0 ± 0.0a	76.0 ± 9.9b	99.0 ± 1.0ab
2016	0	5	1.6 ± 0.4a	0.3 ± 0.1b	0.8 ± 0.1a	53.8 ± 8.9	21.3 ± 4.2	37.5 ± 3.0
	7	12	2.4 ± 0.9a	0.3 ± 0.1b	2.2 ± 1.4ab	46.9 ± 6.3a	18.1 ± 5.2b	43.1 ± 8.1ab
	16	21	3.8 ± 0.9a	0.5 ± 0.2b	3.3 ± 1.3a	50.0 ± 4.9	27.5 ± 5.1	44.4 ± 7.2
	21	26	6.3 ± 2.0a	1.7 ± 0.2b	5.7 ± 1.4ab	68.8 ± 4.7	54.4 ± 3.2	58.1 ± 5.5
	34	39	24.4 ± 2.5a	6.6 ± 1.2b	10.8 ± 3.9ab	79.4 ± 2.7	81.9 ± 6.5	76.3 ± 4.4
2017	0	0	0.3 ± 0.1	0.8 ± 0.3	1.2 ± 0.4	8.8 ± 2.1	17.5 ± 3.8	19.4 ± 6.0
	7	7	4.6 ± 0.7a	1.4 ± 0.1b	1.7 ± 0.5ab	63.1 ± 2.1a	40.6 ± 2.0b	44.4 ± 6.9ab
	14	14	6.3 ± 0.7a	1.8 ± 0.2b	2.8 ± 0.6ab	51.9 ± 1.9	46.3 ± 1.8	48.1 ± 0.8
	24	24	22.5 ± 2.9a	5.9 ± 2.4b	6.0 ± 1.5b	70.6 ± 3.2	68.8 ± 4.1	62.5 ± 4.0
	31	31	17.6 ± 1.7a	5.0 ± 0.4b	6.3 ± 2.2ab	68.1 ± 3.5b	86.3 ± 1.3a	57.5 ± 4.0b
	37	37	18.6 ± 2.4a	6.5 ± 0.9b	8.9 ± 2.4ab	75.6 ± 2.7b	91.3 ± 2.3a	80.0 ± 4.0ab
	43	43	20.5 ± 2.5a	12.2 ± 1.6b	7.9 ± 1.4b	70.0 ± 4.3b	85.6 ± 1.3a	70.6 ± 4.3b

Downy mildew severity and incidence were assessed in the validation trial on leaves of cucumber plants after foliar (2015 and 2016) or root (2017) application of 0.8% tagatose as formulated product (0.8% F_TAG). Disease assessment was carried out in the autumn trial and tagatose was combined with sulphur in the 2016 trial. As control, plants were left untreated (CTRL) or treated with chemical products commonly used for powdery mildew control (chemical treatment, Table 1). Mean and standard error values of three (2015) and five (2016 and 2017) replicates (eight plants each) are reported for each treatment. For each trial, different letters indicate significant differences among treatments at each time point, according to the Kruskal-Wallis ($P \leq 0.05$). Letters were omitted for time points with no significant differences among treatments.

Validation, Resources, Writing - Review & Editing, Project Administration. SN: Formal Analysis, Investigation, Visualization. OG: Methodology, Investigation. AN: Methodology, Investigation, Resources. MP: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision, Project Administration. IP: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition.

All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2021.105753.

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